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# Salt-independent binding of antibodies from human serum to thiophilic heterocyclic ligands

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**Abstract**

Several thiophilic adsorbents with mercaptoheterocyclic ligands have been analyzed for their ability to bind human serum proteins in a salt-independent way. In contrast to 2-mercaptopyrimidine, 2-mercaptopyridine derived ligands show a group-selective binding of immunoglobulins and $\alpha_2$-macroglobulin, not only in the presence of high concentrations of sodium sulphate but in buffers with low ionic strength. The binding is restricted to thiophilic gels obtained by coupling 2-mercaptopyridine to a vinylsulphone-activated matrix and is not achieved on epichlorohydrin-activated gels. A novel thiophilic ligand based on mercaptonicotinic acid, containing a carboxylic group together with the thiophilic pattern of thioaromatic adsorbents, is demonstrated to be useful as an alternative purification scheme for antibodies. 

*Keywords:* Antibodies; Proteins; 2-Mercaptopyridine; 2-Mercaptopyrimidine

## 1. Introduction

In 1985 Porath et al. [1] introduced a new chromatographic method; thiophilic adsorption chromatography. For this, SH-containing ligands, originally 2-mercaptoethanol, are immobilized on divinylsulphone-activated agarose [1,2], glass [3] or nylon [4] producing the so called thiophilic (T) adsorbents which exhibit a salt-promoted selective adsorption of proteins by interacting with, as yet poorly identified, thiophilic regions of such proteins. In the case of human serum proteins it has been shown that the T-gels are particularly selective for immunoglobulins and $\alpha_2$-macroglobulin ($\alpha_2$M) [1]. Both aliphatic as well as $\pi$ electron-rich aromatic or heteroaromatic ligands have been studied. Originally, the sulphone group and the thioether structure seemed to be essential for the characteristic protein binding behaviour of the thiophilic gels. However, a similar group-specific adsorption of antibodies from different sources was observed by coupling heterocyclic ligands to epoxy-activated matrices [3,5]. To distinguish between the two general groups of adsorbents directed towards thiophilic protein structures, Porath [6] proposed a nomenclature with the designations T-adsorbents (thioether sulphonyl ligands) and thioaromatic T-adsorbents (ligands with the structures R-$SO_2$-$CH_2$– or R-S-$CH_2$-$CH_2$-$SO_2$-, where R is a $\pi$ electron-rich aromatic or heteroaromatic ligand). More recently, however, this

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notation for thiophilic adsorbents has not strictly been adhered to and has also been used for structures others than those listed by Porath [6].

In the view of the wide application of monoclonal as well as polyclonal antibodies in many fields of biotechnology and biomedicine with the need for fast, efficient and rapid purification of antibodies, thiophilic chromatography has turned out to be an alternative method to protein A or protein G affinity purification [4,7–11]. Obviously, synthesis of thiophilic gels is simple and the ligands are of low cost. Conversely, thiophilic chromatography shares with other established antibody purification methods the disadvantage of a labor-intensive sample preparation. For instance, the antibody solutions (serum, cell supernatants, ascites) have to be adjusted to high salt concentrations before being applied to the gels, and often the resulting solutions have to be cleared by filtration or centrifugation in order to avoid blocking of gel flow by particles. Interestingly, there are reports of a salt-independent binding of proteins to thiophilic gels. Hutchens and Porath [2] found the salt-independent adsorption of five model proteins to be different, pH-dependent and easily reversed by salt and considered it as a relatively minor interaction event and therefore likely a separate adsorption property of the thiophilic ligand or even the divinylsulphone modified agarose itself.

This study was done using mercaptoethanol as thiophilic ligand and purified proteins. Immunoglobulins were among the three proteins with a salt-independent adsorption tendency, but their adsorption was prevented by low concentrations of sodium chloride. Other results with different thiophilic ligands and complex protein mixtures also indicate that thiophilic gels are able to bind proteins in a group-specific manner in the absence of water-structure-forming salts. It seems especially noteworthy that in some cases proteins adsorbed at high concentrations of water-structure-forming salt are not completely desorbed by decreasing the salt amount in the buffer systems [3,10]. However, it has never been tried to take advantage of this binding property of thiophilic adsorbents for the purification of proteins. We, therefore, investigated in more detail the salt-independent binding of human serum proteins to agarose substituted with mercaptoheterocycles.

## 2. Experimental

### *2.1. Reagents and chemicals*

Sodium sulphate, sodium carbonate, sodium chloride, buffer phosphates, 2-mercaptoethanol and sodium hydroxide were purchased from Sigma (Deisenhofen, Germany). 2-Mercaptopyridine, 2-mercaptopyrimidine, 2-mercaptonicotinic acid, sodium borohydride, epichlorohydrin and vinylsulphone were from Aldrich (Steinheim, Germany), Sepharose 4B-CL from Pharmacia (Uppsala, Sweden), glycerol and ethanol from Merck (Darmstadt, Germany).

### *2.2. Serum samples*

Normal human blood was obtained from the Blood Bank at the University of Leipzig. Serum was separated from the clot formed in the blood after 30 min at room temperature by centrifugation for 15 min at 6000 *g*. The sera of 12 patients were mixed and this pool were used throughout all the experiments. Total protein, immunoglobulin G (IgG) and $\alpha_2$M were determined to 124.2 mg/ml, 15.4 mg/ml and 2.5 mg/ml, respectively.

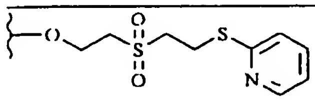
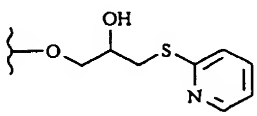
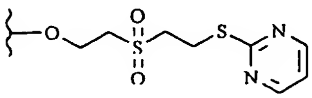
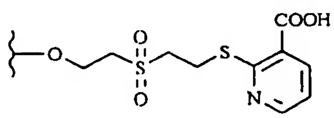
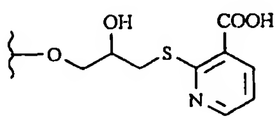
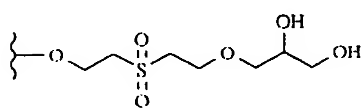
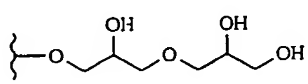
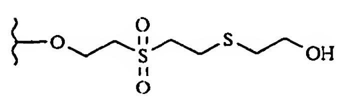
### *2.3. Preparation of the affinity gels*

#### *2.3.1. Vinylsulphone-activated gels*

A 40-g quantity of thoroughly washed and suction-dried Sepharose 4B-CL were suspended in 100 ml of 0.5 *M* sodium carbonate. The suspension was stirred and 4 ml of divinylsulphone were added. After reaction for 2 h at room temperature, the gel was collected on a sintered-glass filter and washed with water until the filtrate was neutral. The activated gel was stored in water at 4°C until use (not longer than one week).

For coupling of the mercaptoheterocycles, 2 g of suction-dried vinylsulphone-activated Sepharose, prewashed with 0.5 *M* sodium carbonate solution, were mixed with 150 μmol of the respective mercaptoheterocycle (Table 1) which were dissolved in 2 ml of 0.5 *M* NaOH. The mixture was shaken for 1 h and then 0.5 ml of glycerol was added. After a reaction time of 6 h the gel slurry was transferred to

Table 1
Structure of thiophilic ligands and designation of the respective gels

| Structure | Gel-designation (ligand/coupling reagent) |
|---|---|
|  | Py-S-Sulphone (2-mercaptopyridine/divinylsulphone) |
|  | Py-S-Hyprop (2-mercaptopyridine/epichlorohydrine) |
|  | Pym-S-Sulphone (2-mercaptopyrimidine/divinylsulphone) |
|  | Nic-S-Sulphone (2-mercaptonicotinic acid/divinylsulphone) |
|  | Nic-S-Hyprop (2-mercaptonicotinic acid/epichlorohydrine) |
|  | control I (no ligand/divinylsulphone) |
|  | control II (no ligand/epichlorohydrine) |
|  | T-gel (2-mercaptoethanol/vinylsulphone) |

a glass filter, washed with water and resuspended in 25 m$M$ sodium phosphate buffer, pH 7.4, containing 0.1% $NaN_3$. The gels were stored at 4°C until use.

As a control, a classical T-gel was synthesized by coupling 2-mercaptoethanol instead of a mercaptoheterocycle to the divinylsulphone-activated Sepharose.

### 2.3.2. *Epoxy-activated gels*

A 20-g sample of thoroughly washed and suction-dried Sepharose 4B-CL and 38 mg sodium borohydride were suspended in 10 ml of 4 $M$ NaOH. A 5-ml volume of 1-chloro-2,3-epoxypropane was added to the mixture and the suspension stirred at room temperature for 19 h. The slurry was washed

with water on a sintered-glass filter until the filtrate was neutral. The epoxy-activated gel was resuspended in 6.25 ml of 50% (v/v) methanol. Afterwards, the heterocycle was added and the suspension stirred for 8 h at room temperature. The gel was then washed with 50% (v/v) methanol, water and then resuspended in 25 m$M$ sodium phosphate buffer, pH 7.4, containing 0.1% $NaN_3$. The gels were stored at 4°C until use.

Control gels were obtained by omitting the mercaptoheterocycles in the coupling reactions.

The gels and their designations used are listed in Table 1.

### 2.4. Chromatography

For chromatography, a column (5×0.5 cm) was packed with the test gel to obtain a bed volume of 1 ml at a buffer flow of 1 ml/min. The column was equilibrated with the starting buffer: 25 m$M$ sodium phosphate, pH 7.4 in the case of salt-free adsorption or 25 m$M$ sodium phosphate, pH 7.4, containing 0.5 $M$ sodium sulphate for the salt-promoted adsorption, respectively. Human serum dialyzed twice against two volumes of the equilibration buffer was spun at 15 000 $g$ and loaded onto the gel. Unbound proteins were removed by washing the column with equilibration buffer. Desorption of proteins bound was achieved by 10 m$M$ NaOH (salt-independent adsorption) or by consecutive washings with 25 m$M$ sodium phosphate, pH 7.4 and 10 m$M$ NaOH (salt-promoted adsorption). Chromatographic separations were run at room temperature, effluents were collected in 1-ml fractions. For each run the fractions were pooled in order to have the non-adsorbed fraction and the eluted fractions in a volume of 10 ml and 5 ml, respectively. The recoveries of the analyzed proteins in these pools were all above 90%.

### 2.5. Protein determination

Protein concentration was determined according to Bradford [12] by the Coomassie Blue dye binding assay using the Bio-Rad protein assay (Bio-Rad, Munich, Germany) with bovine plasma gamma globulin as protein standard. Protein content of the human serum pool was overestimated with the standard used to 124.2 mg/ml.

### 2.6. Polyacrylamide gel electrophoresis

For the characterization of the protein species separated by the chromatography, 0.25 ml of serum and the pooled fractions indicated are each diluted to 20 ml with 25 m$M$ phosphate buffer, pH 7.4 and 5 μl of each dilution were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) according to Laemmli [13] under reducing conditions. The Mini-PROTEAN II System of Bio-Rad was used with spacers of 1 mm thickness. Denaturation was done using the sample buffer of Sigma (Steinheim, Germany). Electrophoresis was run for 60 min at 150 V. For calibration, a mixture of standard proteins ($M_r$ $\alpha_2$M, 170 000; β-galactosidase, 116 400; phosphofructokinase, 85 200; glutamate dehydrogenase, 55 600; aldolase, 39 200; triosephosphate isomerase, 26 600 and trypsin inhibitor, 20 100 from the Combithek of Boehringer, Mannheim, Germany) was separated on the same gel. The gel was stained for 30 min with Coomassie Brilliant Blue G-250 (0.25% in acetic acid–methanol–water (10:5:85)) with gentle shaking. Destaining was performed in acetic acid–methanol–water (10:5:85).

### 2.7. Determination of IgG and $\alpha_2M$

Total IgG and $\alpha_2$M were determined by enzyme immunoassay using the IgG- and $\alpha_2$M enzyme-linked immunosorbent assay (ELISA) kits of Immundiagnostik (Bensheim, Germany). The detection limits of these assays were 0.5 μg/ml and 0.4 μg/ml, respectively.

## 3. Results and discussion

2-Mercaptopyridine [3,5] and 2-mercaptopyrimidine [3] as thiophilic ligands have been successfully applied to the purification of antibodies from complex protein solutions by salt-promoted adsorption. Interestingly, these compounds show a different behaviour in their chromatographic performance. The mercaptopyridine structure was less efficient because at identical chromatographic conditions with modified silica the recovery of a monoclonal antibody was significantly lower compared to

the other heterocycle [3]. This is manifested by the higher amount of sodium sulphate required for complete adsorption of immunoglobulin to the respective thiophilic gels. No significant differences of the behaviour of the two mercaptoheterocycles were observed on silica or agarose activated by glycidyloxypropyltrimethoxysilane or epichlorohydrin, respectively.

A further distinct property of 2-mercaptopyridine is recognizable when it is coupled to vinylsulphone-activated Sepharose (Table 2). As expected, both the Pym-*S*-Sulphone and the Py-*S*-Sulphone gel bind human serum proteins in a salt-promoted way. At 0.5 *M* sodium sulphate, 1.99 mg and 4.81 mg of protein are adsorbed to the respective gel and can be desorbed in two fractions by omitting the sodium sulphate in the buffer and a consecutive wash with sodium hydroxide. However, in contrast to the Pym-*S*-Sulphone gel, significant amounts of human serum proteins (1.96 mg) remain adsorbed to the 2-mercaptopyridine containing thiophilic gel after washing the gel with sodium sulphate-free buffer. This protein has to be desorbed by dilute alkaline and represent about 40% of the total protein adsorbed. Analysis of the protein species present in this fraction revealed that the major part of this protein is represented by immunoglobulins and $\alpha_2$M (Table 3). Remarkably, these proteins are among those with the highest selectivity for the salt-dependent adsorption to thiophilic gels [1]. Even if taken into account that the ELISA technique and the total protein determination method give rise to different protein values, the fraction desorbed from the Py-*S*-Sulphone gel has a higher IgG/total protein ratio than the fraction desorbed by eliminating the sodium sulphate from the buffer. As shown in Table 2, protein binding also occurred to the same extent when serum was applied to the Py-*S*- Sulphone gel in buffer in the absence of sodium sulphate. Here too, the most abundant proteins bound and desorbed by sodium hydroxide are IgG and $\alpha_2$M (Table 3 Fig. 1 lane 3). The electrophoretic analysis reveals, however, some unidentified accompanying proteins. No significant differences in the chromatographic behaviour of human serum proteins are observed if other buffers, for instance Tris, HEPES, imidazole, are used instead of phos-

Table 2
Distribution of human serum proteins in the chromatographic fractions of the thiophilic gels

| | Py-*S*-Sulphone | Pym-*S*-Sulphone | Nic-*S*-Sulphone | Py-*S*-Hyprop | Pym-*S*-Hyprop | Nic-*S*-Hyprop |
|---|---|---|---|---|---|---|
| Chromatography in the presence of 0.5 *M* $Na_2SO_4$ in 25 m*M* Na-phosphate, pH 7.4 | | | | | | |
| Non-adsorbed fractions | 24.85 | 26.27 | 26.85 | 27.15 | 28.17 | 28.89 |
| Desorbed by deleting $Na_2SO_4$ | 2.85 | 1.79 | 1.04 | 1.32 | 1.66 | 0.13 |
| Desorbed with NaOH | 1.96 | 0.20 | 1.54 | 0.08 | 0.06 | 0.07 |
| Chromatography in 25 m*M* Na-phosphate, pH 7.4 | | | | | | |
| Non-adsorbed fractions | 27.58* | 30.88 | 27.74* | 29.44 | 29.85 | 29.54 |
| Desorbed with NaOH | 2.13* | 0.18 | 2.18* | 0.18 | 0.13 | 0.10 |
| Chromatography in 25 m*M* Na-phosphate, pH 7.4 | | | | | | |
| Non-adsorbed fractions | 28.80 | 29.57 | 27.20* | | | |
| Desorbed with 0.5 *M* NaCl | 0.04 | 0.06 | 1.79* | n.d. | n.d. | n.d. |
| Desorbed with NaOH | 1.45 | 0.04 | 0.28* | | | |
| Chromatography in 25 m*M* Na-phosphate, pH 7.4 | | | | | | |
| Non-adsorbed fractions | 28.90 | | | | | |
| Desorbed with 0.5 *M* $Na_2SO_4$ | 0.07 | n.d. | n.d. | n.d. | n.d. | n.d. |
| Desorbed with NaOH | 1.76 | | | | | |

Chromatographies were performed as described in Section 2.4: 0.25 ml of serum were applied to 1 ml of the respective gel and proteins bound desorbed as indicated. The respective fractions were pooled and assayed for protein (n.d.=not determined). Values are the total protein amounts (mg) in each pool. Fractions labeled by an asterisk are analysed by SDS–PAGE (see Fig. 1).

Table 3
Distribution of IgG and $\alpha_2$-macroglobulin in the chromatographic fractions of the thiophilic gels

| | Py-*S*-Sulphone | | Pym-*S*-Sulphone | | Nic-*S*-Sulphone | |
|---|---|---|---|---|---|---|
| | IgG (mg) | $\alpha_2$M (mg) | IgG (mg) | $\alpha_2$M (mg) | IgG (mg) | $\alpha_2$M (mg) |
| Chromatography in the presence of 0.5 *M* $Na_2SO_4$ in 25 m*M* Na-phosphate, pH 7.4 | | | | | | |
| Non-adsorbed fractions | 0.95 | 0.23 | 0.84 | 0.14 | 1.12 | 0.41 |
| Desorbed by deleting $Na_2SO_4$ | 1.26 | 0.21 | 2.95 | 0.35 | 1.51 | 0.13 |
| Desorbed with NaOH | 1.91 | 0.14 | 0.05 | 0.01 | 1.55 | 0.15 |
| Chromatography in 25 m*M* Na-phosphate, pH 7.4 | | | | | | |
| Non-adsorbed fractions | 1.34 | 0.62 | 3.87 | 0.69 | 1.89 | 0.66 |
| Desorbed with NaOH | 2.29 | 0.12 | 0.11 | 0.04 | 1.98 | 0.10 |
| Two consecutive re-chromatographies in 25 m*M* Na-phosphate, pH 7.4 of non-adsorbed fractions | | | | | | |
| Non-adsorbed fractions | 0.11 | 0.59 | | | | |
| Desorbed with NaOH (pool of all fractions) | 3.84 | 0.13 | | | | |

Chromatographies were performed as described in Section 2.4 and Table 2. The respective fractions were pooled and assayed for IgG and $\alpha_2$-macroglobulin ($\alpha_2$M). Values are in mg in each pool.

phate buffer (data not shown). Only traces of proteins were adsorbed in salt-free buffer to the mercaptopyrimidine-substituted thiophilic gels, irrespective of the coupling procedure (Tables 2 and 3). To test whether the immunoglobulins present in the run-through fraction of the Py-*S*-Sulphone gel chromatography represent a distinct group among the antibody population two consecutive chromatographic runs were performed with the non-adsorbed proteins (Table 3). IgG is extracted nearly completely from the run-through fraction after the third chromatography, indicating that the binding is not restricted to

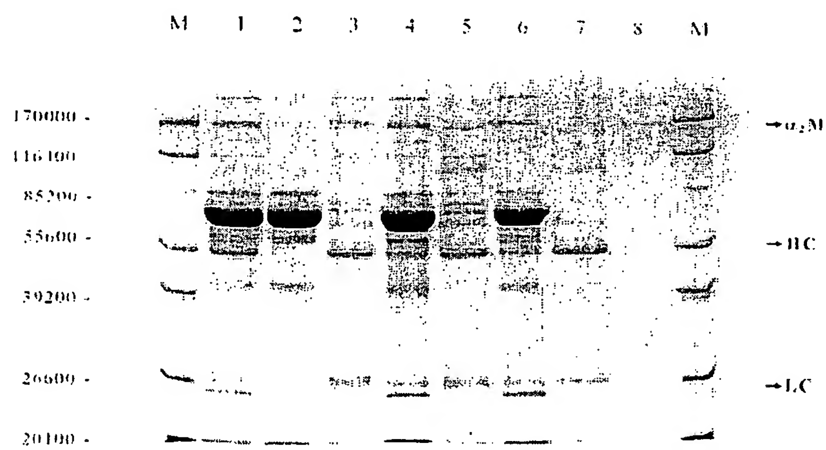



Fig. 1. Human serum protein distribution in the effluents and eluates after adsorption chromatography with Py-*S*-Sulphone and Nic-*S*-Sulphone gels. SDS–PAGE run of the fractions indicated in Table 2: lane M, marker proteins; lane 1, whole blood serum; lane 2, Py-*S*-Sulphone gel effluent; lane 3, Py-*S*-Sulphone gel NaOH eluate; lanes 4 and 6, Nic-*S*-Sulphone gel effluents; lanes 5 and 8, Nic-*S*-Sulphone gel NaOH eluates; lane 7, Nic-*S*-Sulphone gel NaCl eluate. The positions of $\alpha_2$M and the heavy (HC) and light (LC) chains of IgG are marked by an arrow at the right. The molecular masses of the marker proteins are indicated on the left.

some of the heterogeneous IgG molecules. Interestingly, the fraction of $\alpha_2$M which did not adsorb to the gel in the first run remained in the non-adsorbed fraction after being rechromatographed. It has yet to be elucidated which fraction of $\alpha_2$M in human serum is able to bind to the Py-*S*-Sulphone gel.

The salt-independent binding of immunoglobulins to mercaptopyridine-substituted agarose is restricted to the gel obtained by coupling the mercaptoheterocycle to vinylsulphone-activated agarose. The Py-*S*-Hyprop gel does not show a significant binding of human serum protein (Table 2) in salt-free buffer. The control experiments with the classical 2-mercaptoethanol-substituted T-gel and the unsubstituted control gels I and II (Table 4) also confirm that the 2-mercaptopyridine moiety is responsible for the salt-independent protein binding to Py-*S*-Sulphone gel and it is not a property of the unliganded but modified matrix. Only the T-gel binds protein at 0.5 *M* sodium sulphate and none of the three control gels are able to adsorb protein in the absence of sodium sulphate. Therefore, the sulphone group and the mercaptopyridine structure with its distinct distribution of electrons and local charges seem essential for the salt-independent selective interaction of immunoglobulins with the Py-*S*-Sulphone gel. It is, however, necessary to mention, that a gel similar to our control gel I was reported to bind immunoglobulins in a salt-promoted way [14] suggesting that the divinylsulphone-activated matrix itself interacts with proteins alike the thiophilic gels. It remains to be established wether the differences in the experimental conditions (for instance the blocking step with glycerol or the different salts used to promote binding) are responsible for the observed distinct results.

In some respect, namely the salt-promoted adsorption mode, thiophilic chromatography resembles hydrophobic interaction chromatography [1,15]. Lihme and Heegaard [15] compared the behaviour of various serum proteins on a traditional hydrophobic matrix (Phenyl-Sepharose) and on a T-gel (mercaptoethanol coupled to vinylsulphone-activated agarose) and found the elution pattern of immunoglobulins and $\alpha_2$M (and others) considerably different with these two matrices. It was concluded that the T-gel is "more hydrophilic" than a hydrophobic matrix which causes a shift in the adsorption process to lower salt concentrations and an elution as a sharper peak for the T-gel. The salt-independent adsorption observed with the mercaptopyridine gel can thus be considered as the maximum shift possible. Because the selectivity of the T-gels ceases with higher salt concentrations [15] and because a biphasic binding dependent on salt concentration was observed with some proteins [2], one might speculate that thiophilic gels bind proteins in two ways: by the group-selective thiophilic interaction and, superimposed, by a traditional hydrophobic interaction. We therefore synthesized a new hydrophilic thiophilic structure by coupling mercaptonicotinic acid to vinylsulphone-activated agarose. Because of the charge of the dissociated carboxylic group attached to the pyridine it is more hydrophilic than the pyridine ligand itself. The resulting Nic-*S*-Sulphone gel behaves as the Py-*S*-Sulphone gel in respect to its ability to adsorb human serum proteins and, in particular, immunoglobulins in a salt-promoted as

Table 4
Binding of human serum proteins to control gels

| | T-gel | Control I | Control II |
|---|---|---|---|
| Chromatography in the presence of 0.5 *M* $Na_2SO_4$ in 25 m*M* Na-phosphate, pH 7.4 | | | |
| Non-adsorbed fractions | 27.14 | 30.12 | 29.56 |
| Desorbed by deleting $Na_2SO_4$ | 3.11 | 0.15 | 0.08 |
| Desorbed with NaOH | 0.08 | 0.06 | 0.08 |
| Chromatography in 25 m*M* Na-phosphate, pH 7.4 | | | |
| Non-adsorbed fractions | 30.44 | 30.22 | 30.41 |
| Desorbed with NaOH | 0.09 | 0.02 | 0.05 |

Experiments were performed as described in Section 2.4 and Table 2. Values are the total protein amounts (mg) in each pool.

well as in the salt-independent manner (Tables 2 and 3 Fig. 1). However, the main part of the protein bound to the gel in sodium sulphate-free buffer is desorbed by 0.5 *M* sodium chloride, while no such desorption is observed with the Py-*S*-Sulphone gel. If the isoelectric point (p*I*) values of the proteins adsorbed are above the buffer pH, such proteins are positively charged and their adsorption might be explained by the action of the Nic-*S*-Sulphone gel as a weak cation exchanger. However, in contradiction to this explanation are the findings that sodium sulphate is not able to desorb a significant part of the bound proteins as is the more chaotropic sodium chloride (Table 2) and that all of the proteins bound to the Py-*S*-Sulphone gel also bind to the Nic-*S*-Sulphone gel when rechromatographed after neutralizing the effluent desorbed by NaOH (not shown). Furthermore, the Nic-*S*-Sulphone gel adsorbs far more protein than the respective hydroxypropyl group-containing gel but is expected to work as an ion exchanger to the same extent as does the Nic-*S*-Hyprop gel. Obviously, this reflects a distinct binding behaviour of the thiophilic structures independent of ionic interaction of the dissociated carboxylic residue.

The selective binding of serum proteins, namely immunoglobulins and $\alpha_2$M, to thiophilic structures in buffers of low-ionic strength provides further evidence for excluding general hydrophobic interactions as the major principle underlying the protein binding to thiophilic gels. The most pronounced salt-independent binding is observed with pyridine-based ligands containing the sulphone group while other thiophilic structures show no or only a very weak interaction in the absence of water-structure forming salt. As demonstrated, it may be used for the purification of antibodies with the advantage that sample preparation is easier because there is no need to adjust the samples to high salt concentrations before the chromatography. The main limitation of the use of the pyridine-based thiophilic structures for the purification of antibodies is their relatively low affinity. To isolate all of the immunoglobulin, chromatography has to be repeated or the amount of gel has to be increased. A systematic screen for other heterocyclic or even aliphatic compounds in combination with a theoretical description of the salt-independent binding phenomenon in terms of charge distribution, $\pi$-electron distribution, p*K* values of dissociable groups may, however, help to find new ligand structures with higher affinity.

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